CLAIMS

[1] A novel mixture or novel reaction solution for assaying one or two or more target nucleic acids, which comprises one or two or more below nucleic acid probes for a homogenous solution system and one or two or more below internal standard nucleic acids, or further one or two or more below internal standard nucleic acid probes:

A) said nucleic acid probe for a homogenous solution system (hereinafter, called a "target nucleic acid probe") having below characteristics :

a) said target nucleic acid probe for a homogenous solution system is formed of one single stranded oligonucleotide;

b) said target nucleic acid probe for a homogenous solution system is labeled with one or two or more molecule of fluorescent dyes of one or two or more kinds at at least one of both end portions and/or at least one of base moieties in the chain, at least one of sugar moieties and/or at least one of phosphate moietes of the oligonucleotide;

c) said target nucleic acid probe for a homogenous solution system enables a fluorescent character to change on hybridizing with a target nucleic acid and/or an internal standard nucleic acid;

d) said target nucleic acid for a homogenous solution system is capable of hybridizing without discriminating with a target nucleic acid or an internal standard nucleic acid;

e) said target nucleic acid probe for a homogenous solution system is capable of producing a difference between the amount of change in a fluorescent character on hybridization with an internal standard nucleic acid and the amount of change in a fluorescent character on hybridization with a target nucleic acid.

B) Said internal standard nucleic acid:

Said internal standard nucleic acid has a structure different in at least a portion from the structure of a region of a target nucleic acid corresponding to the target nucleic acid probe, and said internal standard nucleic acid is capable of causing a difference between the amount of a change in a fluorescent character on hybridizing with the target nucleic acid probe and the amount of a change in a fluorescent character on the hybridization of a target nucleic acid with the target nucleic acid probe.

C) Said internal standard nucleic acid probe:

Said internal standard nucleic acid probe has the following characteristics:

Said internal standard nucleic acid probe has said characteristics a) to e) of the target nucleic acid probe, wherein a fluorescent labeling portion and a fluorescent character of a labeled fluorescent dye each are different from that of the target nucleic acid probe.

[2] A novel method for assaying a target nucleic acid,

comprising assaying one or two or more target nucleic acids using the novel mixture according to claim 1.

[3] A novel method for assaying a target nucleic acid according to claim 2, wherein said novel mixture comprises the below target nucleic acid probe and a predetermined concentration of the internal standard nucleic acid according to claim 1:

said target nucleic acid probe (a recognizable nucleic acid probe), wherein said target nucleic acid probe according to claim 1 is not complementary to either a target nucleic acid or an internal standard nucleic acid at at least one or two or more fluorescent-labeled portions.

[4] A novel method for assaying a target nucleic acid, in which said method comprises quenching an fluorescent emission of the target nucleic acid probe or the internal standard nucleic acid probe not hybridized with either an internal standard nucleic acid or a target nucleic acid in the novel method for assaying a target nucleic acid according to claim 2 or 3.

[5] A novel method for assaying a target nucleic acid, in which comprises: multiplying one or plural target nucleic acids and internal standard nucleic acids in a reaction system comprising one or plural internal standard nucleic acids according to claim 1 until an optional phase of from a beginning phase to a stationary phase by a gene amplification method;

and determining starting concentrations of one or plural target nucleic acids prior to the amplification using the resultant reaction solution or the amplified product as a sample.

[6] A novel mixture for assaying one or plural target nucleic acids based on measurement of a Tm value, in which said mixture comprises a pair of the below nucleic acid and the below internal standard nucleic acid:

Said nucleic acid probe: Said nucleic acid probe is a single stranded oligonucleotide labeled with one or two or more fluorescent dyes, wherein said nucleic acid probe is capable of hybridizing with a target nucleic acid and the below internal standard nucleic acid, and causing changes in fluorescent characters of the fluorescent dyes labeled thereon on hybridization with the target nucleic acid and internal standard nucleic acid, wherein, in the case when said nucleic acid probe is plural, the fluorescent dyes labeled on said plural nucleic acid probes each are different.

Said internal standard nucleic acid: The base sequence of a portion of said internal standard nucleic acid hybridizing with said nucleic acid probe is different in part from the base sequence of a portion of a nucleic acid hybridizing with said nucleic acid probe.

[7] A novel method for assaying a target nucleic acid, in which said novel method comprises measuring fluorescent

intensity using said novel mixture according to claim 6 with changing temperature under the presence of plural target nucleic acids; and determining a target nucleic acid by the following procedures:

Said procedures comprising:

1) drawing a curve dependent to changed fluorescent intensity measured;

2) differentiating the resulting curve;

3) integrating the resulting peak(s) and determining the area(s) of the peak(s);

4) calculating a ratio(s) of the resulting peak area(s) of the internal standard nucleic acid and the resulting peak area(s) of the target nucleic acid;

5) multiplying the concentration of the internal standard nucleic acid by said ratio.

[8] A calculating equation represented by the following equation for calculating accurately a target nucleic acid based on measuring values of a change in an optical character in said novel method for assaying a target nucleic acid according to claims 2 and 3:

$$x=(-a'-B+Ba'+b'+A-Ab')/(b'-b-Ab'+Ab-a'+a+Ba'-Ba)$$

wherein

said equation is under the below conditions; and said signs have the below meanings:

Said conditions are as follows: said novel method uses

the doubly-labeled nucleic acid probe, wherein said nucleic acid probe is labeled with dyes A and B.

Said signs are as follows:

x: a proportion of a target gene;

A: a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on no hybridization in use of a practical sample to the fluorescent intensity of dye A of the doubly-labeled nucleic probe on no hybridization;

a: a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a target nucleic acid to the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on no hybridization;

a': a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with an internal standard nucleic acid to the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on no hybridization;

B: a ratio of the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on hybridization using a practical sample to the fluorescent intensity of dye B of the doubly-labeled nucleic probe on no hybridization;

b: a ratio of the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on its 100%-hybridization with a target gene to the fluorescent intensity of dye B of

the doubly-labeled nucleic acid probe on no hybridization;

b': a ratio of the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on its 100%-hybridization with an internal standard nucleic acid to the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on no hybridization.

[9] A calculating equation represented by the following equation for calculating accurately a target nucleic acid based on measuring values of a change in an optical character in said novel method for assaying a target nucleic acid according to claims 2 and 3:

$$x=(b-B-Ab+A+a'B-a')/(a'B-a'-aB+a)$$

wherein said equation is under the below conditions; and said signs have the below meanings:

Said conditions are as follows: said novel method uses said doubly-labeled nucleic acid probe, wherein said nucleic acid probe is labeled with dyes A and B.

Said signs are as follows:

x: a proportion of a target gene;

A: a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching substance-labeled nucleic acid probe in the case making use of a practical sample to the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching substance-labeled

nucleic acid probe

a: a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a target nucleic acid to the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching substance-labeled nucleic acid probe;

a': a ratio of the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with an internal standard gene to the fluorescent intensity of dye A of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching substance-labeled nucleic acid probe;

B: a ratio of the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching substance-labeled nucleic acid probe in the case making use of a practical sample to the fluorescent intensity of dye B of the doubly-labeled nucleic probe on its 100%-hybridization with a quenching substance-labeled nucleic acid probe;

b: a ratio of the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on its 100%-hybridization with a target gene and an internal standard gene to the fluorescent intensity of dye B of the doubly-labeled nucleic acid probe on its 100%-hybridization with a quenching

substance-labeled nucleic acid probe.

[10] A kit for assaying a nucleic acid, in which said kit comprise the novel mixture according to claim 1 or 6.

[11] A target nucleic acid probe or a doubly-labeled nucleic acid probe, wherein said target nucleic acid probe or doubly-labeled nucleic acid probe is described above and has at least any one of the below structures:

1. Said structures of said target nucleic acid probe, wherein

1) said structure has a portion not complementary to a target nucleic acid and/or an internal standard nucleic acid at a end portion or both end portions;

2) in the above 1), said nucleic acid probe is labeled with a fluorescent dye at one end portion not complementary to the target nucleic acid and/or an internal standard nucleic acid, having a cytosine (a C) or a guanine (a G) in a range of the 1st base to 3rd base from the labeled base in a fluorescent dye-labeled portion (the labeled base is counted as 1st base);

3) in the above 1), the other one end portion not complementary to a target nucleic acid and/or an internal standard nucleic acid is at a portion opposite said one end portion labeled with a fluorescent dye;

4) in the above 1), the other end portion not complementary to a target nucleic acid and/or an internal standard nucleic acid is in a range of one to four bases in a chain length;

5) in the above 1), if any of two bases of a target nucleic acid corresponding to the both ends of the target nucleic acid probes is a G, that of the internal standard nucleic acid is a base other than a G; if that of the target nucleic acid is a base other than a G, that of the internal standard nucleic acid is a G.

2. Structures of said doubly-labeled target nucleic acid probe, wherein

6) portions labeled with fluorescent dyes were at least two bases;

7) the bases according to the above 6) are two C's;

8) the two C's according to the above 7) are the bases of both ends;

9) the base sequence according to the above 6) are complementary to a target nucleic acid or an internal standard nucleic acid excluding both end portion (at least from one base to three bases in chain length from an end base, the end base counted as the 1st base);

10) the doubly-labeled nucleic acid probe according to the above 6) or 9) is a doubly-labeled nucleic acid probe making at one portion a difference between the amount of a change in a fluorescent character on hybridization with a target nucleic acid and that on hybridization with an internal standard nucleic acid, but not making such a difference at the other portion;

3. Structures common to said target nucleic acid probe and said doubly-labeled nucleic acid probe:

wherein

11) in any one of the above 1) to 10), a base sequence of said target nucleic acid probe or a doubly-labeled nucleic acid is at least complementary to a target nucleic acid or an internal standard nucleic acid excluding both end base portions (a base sequence of at least one base to three bases in length; the end base counted as the 1st base);

12) in any one of the above 1) to 11), said target nucleic acid probe or said doubly-labeled nucleic acid probe has a base sequence completely complementary to a target nucleic acid and an internal standard nucleic acid;

13) in any one of the above 1) to 12), if the base of a target nucleic acid corresponding to an end base of a target nucleic acid probe or a doubly-labeled nucleic acid probe is taken as the 1st base, the number of a G of the corresponding target nucleic acid or internal standard nucleic acid in a range of from the 1st base to the 3rd base is larger in a target nucleic acid than in an internal standard nucleic acid, or smaller in a target nucleic acid than in an internal standard nucleic acid;

14) in any one of the above 1) to 13), if the base of a target nucleic acid corresponding to both end base of a target nucleic acid probe or a doubly-labeled nucleic acid

probe is taken as the 1st base, the number of a G of the corresponding target nucleic acid and internal standard nucleic acid in a range of from the 1st base to the 3rd base is larger in a target nucleic acid than in an internal standard nucleic acid in one end region, and in the other end region smaller in a target nucleic acid than or equal in an internal standard nucleic acid in the other end rejoin; or in one end region smaller in a target nucleic acid than in an internal standard nucleic acid and in the other end region larger in a target nucleic acid than or equal in an internal standard nucleic acid;

15) a novel mixture according to claim 1 or 5, wherein, in any one of the above 1 to 14, the base of a target nucleic acid corresponding to one end base of the target nucleic acid probe or the doubly-labeled nucleic acid probe is a base other than a G and the base corresponding to the other end base is a G.

16) in any one of the above 1) to 15), if any of two bases of a target nucleic acid corresponding to both end bases a target nucleic acid probe is a G, that of an internal standard nucleic acid is a base other than a G; and if that of a target nucleic acid is a base other than a G, that of an internal standard nucleic acid is a G;

17) in any one of the above, if the base of a target nucleic acid corresponding to both end base of a target nucleic acid

probe or a doubly-labeled nucleic acid probe is taken as the 1st base, the corresponding base sequence of a target nucleic acid and internal standard nucleic acid is different in one end region in a range of from the 1st base to the 3rd base, but the same in the other end region;

18) in any one of the above, if the base of a target nucleic acid corresponding to both end base of a target nucleic acid probe or a doubly-labeled nucleic acid probe is taken as the 1st base, the corresponding base sequence of a target nucleic acid and internal standard nucleic acid in a range of from the 1st base to the 3rd base is different in both end region.